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Secondary Abnormalities of Chromosome 6q in B-Cell Chronic Lymphocytic Leukemia: A Sequential Study of Karyotypic Instability in 51 Patients

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Although karyotypic abnormalities are well documented in B-cell chronic lymphocytic leukemia (B-CLL), few sequential cytogenetic studies have been done. In this study, peripheral blood lymphocytes from fifty-one patients with B-CLL were sequentially karyotyped over a mean interval of 13.8 months (range, one to 51 months). Cytogenetic clones were detected in 33/51 patients (66%) on initial study, including 17 patients with structural abnormalities of chromosome 13q14, and three patients with trisomy 12. Karyotypic evolution was documented in 22/51 patients (43%). The most common secondarily acquired chromosome aberrations were structural abnormalities of the long arm of chromosome 6 involving the region of 6q21-q24 (six patients). Four patients each had acquired structural abnormalities of 1q, 3p, 12q, and 13q. Disease progression, as measured by advance in Rai stage or death from the disease, was observed more often in the clonal evolution group than in the karyotypically stable group (11/22 vs. 5/29; *P* = 0.017). Patients with secondary abnormalities of 6q had a significantly decreased progression-free survival interval compared with other patients in the study (*P* = .023). The authors conclude that clonal karyotypic evolution is common in B-CLL, and that clonal evolution correlates with clinical disease progression. Furthermore, the poor outcomes previously attributed to CLL with 6q abnormalities may be related to the clonal acquisition of these abnormalities over time. Future studies should focus on the relevant genetic events underlying the clinical progression observed with karyotypic evolution of B-CLL. Am. J. Hematol. 59:223–229, 1998.

Key words: chronic lymphocytic leukemia; cytogenetics; clonal evolution; chromosome 6

INTRODUCTION

Clonal karyotypic abnormalities are present in over half of the reported cases of B-cell chronic lymphocytic leukemia (B-CLL) [1,2]. Cohort studies have demonstrated adverse clinical outcomes in B-CLL patients with complex karyotypes compared to those with isolated chromosome abnormalities or normal karyotypes [2]. When single cytogenetic abnormalities are examined, trisomy 12 is associated with shortened survival, whereas isolated abnormalities of chromosome 13q14 are associated with favorable outcomes, comparable to those of patients lacking clonal chromosome abnormalities [2]. Previous studies suggest that the morphologic and immunophenotypic variation observed in B-CLL is also related to karyotype [3–7].

B-CLL was once considered a slowly progressive, genetically stable disease [8,9]. Data characterizing patterns of clonal evolution in B-CLL are scarce. In fact, the largest previously published study of sequential karyotypes in B-CLL included only 18 patients with documented clonal evolution [10]. Furthermore, data from studies correlating karyotypic evolution in B-CLL with clinical disease course have been inconsistent [8–12].

In a retrospective analysis of previously karyotyped B-CLL cases, we identified 51 for whom sequential cy-

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togenetic data were available. Our goal was to characterize the spectrum of secondarily acquired karyotypic abnormalities in this group, and to evaluate these cytogenetic changes in the context of clinical disease course.

MATERIALS AND METHODS

Fifty-one patients with B-CLL were included in the study. Initial karyotypes for 50 of the subjects were published elsewhere [1], but the sequential cytogenetic data have not yet been reported. In the current study, patients 1 through 50 directly correspond to patients 1–5, 7, 8, 10–15, 17, 18, 20, 22, 24, 26, 27, 30, 32, 33, 35, 39, 41, 43, 45, 46, 48–50, 52, 54–58, 62–65, 68, 69, 70, 71, 73, 77, 80, and 81, respectively, of the report by Peterson et al. [1]. Patient no. 51 in the current study has not been reported previously; this patient had normal karyotypes at baseline and at nine-month follow-up.

Wright-Giemsa stained blood smears were examined for all patients. All patients had B-cell clonal origin documented by either immunophenotypic study, or by the presence of clonal antigen receptor gene rearrangements [1]. Based on the review of each available peripheral blood smear, each patient was diagnosed as either CLL, or CLL with >10% circulating prolymphocytes (CLL/PL) [13].

Each patient in the study had at least two, and at most five, separate cytogenetic studies performed (mean 2.5). The time interval between the baseline cytogenetic analysis and the final analysis ranged from one to 51 months (mean 13.8 months). Cytogenetic analysis was performed using buffy coat preparations of peripheral blood. In a single case (patient no. 20), only bone marrow was used for cytogenetic study. The cytogenetic methods used in this study were described in detail elsewhere [1]. Briefly, each buffy coat sample was cultured for 3–4 days at 37°C in 10 ml of RPMI medium with 16% fetal bovine serum, one drop of 1,000 U/ml sodium heparin, 0.6 mg/ml glutamine, 20 U/ml penicillin, and 20 μg/ml streptomycin. B-cell mitogens were also included in each culture. The specific mitogen used varied among different samples, and included one of the following: Phorbol 12-myristate 13-acetate (TPA) (0.2 mg/ml); phorbol 12, 13 dibutyrate (PDBu) (0.005 μg/ml); pokeweed mitogen (PWM) (0.01 mg/ml); or a mixture of PWM (0.01 mg/ml) and lipopolysaccharide (LPS) (0.08 mg/ml). Colcemid (0.07 μg/ml) was added 30 min prior to harvest. A minimum of 15 metaphases were evaluated for each sample. Karyotypes were prepared and analyzed according to the International System for Human Cytogenetic Nomenclature (ISCN) guidelines [14]. Clonal loss of the Y chromosome was considered a clonal cytogenetic abnormality for purposes of analysis, even though loss of the Y chromosome can be a constitutional finding in elderly patients.

Follow-up clinical staging data were available for all 51 patients. Clinical stage was assigned according to Rai et al. [15]. Clinical disease progression was defined as an advance in Rai stage since the last follow-up, or death from B-CLL. The initial stage for each patient was recorded as the stage at the time of initial karyotype. Follow-up staging was evaluated only when a simultaneous karyotype was available for analysis. Death from disease was observed in two patients (patients no. 10 and no. 20), and was recorded as disease progression despite the lack of available karyotype at the time of death. Patient no. 10 died 11 months after the last karyotype, and patient no. 20 died one month after the last karyotype. Karyotypic evolution was observed in both of these patients.

Statistical analyses were performed using SPSS software, version 6.1.2 (SPSS, Chicago, IL). The relationship between clinical disease progression and the presence or absence of clonal evolution was examined using the Fisher's exact test. The effect of prior therapy on clonal evolution was also examined by Fisher's exact test. Likewise, analysis of the differences in follow-up intervals between clonally evolved and nonevolved patients were done using the Student's *t*-test for independent variables. Progression-free survival intervals were examined using Kaplan-Meier product limit curves, and differences in progression-free survival were analyzed using the log-rank test.

RESULTS

Baseline Clinical Data

Forty-nine of the 51 patients (96%) were male, and two (4%) were female. At the time of the initial study, patients ranged in age from 54 to 76 years, with a mean age of 66. Twenty-nine of the 51 patients (57%) had received prior chemotherapy. At the time of the initial study, 17 patients were Rai stage 0, eight patients were stage I, 12 patients were stage II, six patients were stage III, and seven patients were stage IV. For the one remaining patient (patient no. 10), initial staging data were unavailable, and the patient died of B-CLL one month after a 15-month follow-up study was performed.

Baseline Cytogenetic Studies

Thirty-three of the 51 patients (65%) had clonal cytogenetic abnormalities (published previously in detail [1]) at the time of the baseline analysis. Structural abnormalities of chromosome 13q14, the most common clonal chromosome abnormality at baseline, were seen in 17 of the 33 patients (52%) with clonal abnormalities. Trisomy 12, the next most-common recurrent abnormality at baseline, was observed in three patients (9%).

Eighteen of the 33 patients with clonal cytogenetic abnormalities at baseline (55%) had complex karyotypes with more than one clonal abnormality; this included 13

patients with multiple clones or subclones at the time of initial study. The remaining fifteen patients (45%) had only single, isolated cytogenetic abnormalities. These isolated abnormalities included six cases of structural abnormalities involving chromosome 13q14 [three deletions and three translocations—t(11;13)(p15;q14), t(11;13)(q21;q14), and t(13;18)(q14;p11)]. The remaining nine isolated abnormalities were divided among trisomy 12 (two cases), addition of a Y chromosome (one case), loss of the Y chromosome (one case), structural deletions involving chromosomes 6q15-q24, 9p12-pter, and 11p14-pter (one case each) and two additional translocations, t(9;12)(p34;p11) and t(9;14)(p12;q32).

Follow-up Cytogenetic Studies

Karyotypic evolution occurred in 22 of the 51 patients (43%) (Table I). Seven of these 22 patients (32%) had normal karyotypes at baseline, but developed clonal abnormalities over time. The majority of secondarily acquired chromosome abnormalities were structural (Table II). The most common secondarily acquired structural abnormalities were translocations or loss of material involving the long arm of chromosome 6 in the region of 6q21-6q24. These abnormalities occurred in six patients and included one dicentric (6;17)(q21;p13) (patient no. 8), one derivative (6)t(6;15)(q21;q21) (patient no. 11), one deletion (6)(q15q24) (patient no. 13), one deletion (6)(q21q24) (patient no. 24), one deletion (6)(q12q26) (patient no. 32), and one derivative (6)t(2;6)(q24;q24) (patient no. 42) (Table I). Structural abnormalities of chromosome 13q14 were observed as a secondarily acquired abnormality in two patients (Table II), both of whom had normal karyotypes at baseline. Structural abnormalities of chromosome 11q were seen as secondary abnormalities in three patients. A fourth patient acquired a nonclonal abnormality involving chromosome 11q (Table II).

There was no difference in clonal karyotypic evolution between patients with normal karyotypes at baseline and patients with abnormal baseline karyotypes (7/18 vs. 15/33; $P = 0.77$).

Six of the 29 patients in whom karyotypic evolution was not observed had complex karyotypes at baseline analysis. In five of these patients the clones did not share common abnormalities. In one of these patients (patient no. 3) the baseline karyotype contained multiple cytogenetically related clones, suggestive of prior cytogenetic evolution. This patient did exhibit disease progression, but he was included in the nonevolution group for analysis.

Because the incidence of clinical disease progression was potentially a function of the length of the follow-up intervals, we compared these intervals between groups. The elapsed time from baseline cytogenetic study to either a change in karyotype or to final analysis did not differ significantly between the groups with and without karyotypic evolution (14.4 months vs. 12.6 months; $P = 0.26$).

There was no difference in the incidence of karyotypic evolution between previously untreated patients and patients who had received prior therapy (8/19 vs. 12/29).

Clinical Correlation With Clonal Karyotypic Evolution

Clinical disease progression (advance in Rai stage, or death from disease) was observed in 11 of the 22 patients (50%) with karyotypic evolution, and in five of the 29 patients (17%) without karyotypic evolution ($P = 0.017$) (Figure 1).

Progression-free survival intervals were shorter in the group of patients exhibiting karyotypic evolution (median 16 months) than in the karyotypically stable group (median 24 months), but this difference was not statistically significant ($P = .096$) (Figure 2). Patients with secondarily acquired abnormalities of chromosome 6q had significantly shorter progression-free survival intervals (median 16 months) than the remaining patients in the study (median 24 months) ($P = .023$) (Figure 3), although the overall incidence of eventual disease progression was no different between the two groups ($P = .64$). No difference in progression-free survival interval was noted with any other single secondary abnormality.

DISCUSSION

This sequential study of 51 B-CLL patients included 22 (43%) in whom karyotypic instability was noted over time. The majority of temporally acquired karyotypic changes were structural, and included deletions, additions, and translocations involving a variety of different chromosomes (Table I). Whereas no consistent pattern of clonal evolution was observed, the most common secondary chromosome abnormalities involved the long arm of chromosome 6 in the region of 6q21-q24. Structural chromosome 6 abnormalities have been described previously in B-CLL and other lymphoid malignancies [16–18], but they have not been documented previously as common secondarily acquired abnormalities in the karyotypic evolution of CLL. All but one of the temporally acquired 6q abnormalities arose in patients with other clonal abnormalities detectable at baseline, supporting the occurrence of clonal evolution in these cases.

Although there are scattered reports of sequential cytogenetic analyses in B-CLL, karyotypic evolution in B-CLL has not been widely studied. Early longitudinal studies supported the concept of B-CLL as a cytogenetically stable disease [8,9]; however, subsequent reports demonstrated karyotypic changes over time [10,12]. In the largest of such studies, Oscier and coworkers [10] documented karyotypic evolution in 18 of 112 patients

TABLE I. Summary of Clinical and Karyotypic Features in the 22 Patients With Karyotypic Evolution*

Patient no.	Prior therapy	Baseline Rai stage	Baseline karyotype	Follow-up Rai stage	Follow-up karyotype[a]	Time to disease progression (months)
1	Yes	0	46, XY, del (11)(?q21q22), del (13)(?q13?q31), der(16)t(16;17)(q24;q21)[12]	3	46, XY, del (11)(?q21q22), del (13)(?q13q31), der(14)t(X;14)(q13;q32), der(16)t(16;17)(q24;q21), add(17)(q23)[20][b]	9
2	No	2	45, X, −Y[2]/ 45, X, −Y, del(13)(q13q14)[9]/ 46, XY[9]	4	45, X, −Y, del(13)(q13q14)[10]/ 45, idem, t(1;10)(q21;q24)[4][b]	19
4	Yes	1	46, XX, dup(5)(q33q13)[6]/ 46, XX, idem, der(14)t(5;14)(q33;q32)inv(5)(q33q13)[6]/ 46, XX, idem, dup(5)(q33q13)[3]/ 45, idem, −13, der(18)t(13;18)(q12;p11)[3]/ 46, idem, −3, der(16)t(3;16)(q21;q24), +r[3]/ 46, XX[2]	3	46, XX, dup(5)(q33q13)[7]/ 46, idem, dup(5)(q33q13)[4]/ 45, idem, −13, der(18)t(13;18)(q12;p11)[4]/ 45, idem, −18, add(19)(p13)[4][b]/ 46, idem, der(14)t(5;14)(q33;q32)inv(5)(q33q13)[2]/ 45, idem, der(18)t(18;22)(p11;q11), −22[2][b]/ 46, XX[2]	13
6	Yes	3	46, XY, der(17)t(17;17)(p11;q21)[4]/ 45, idem, dic(13;14)(p13;p13)inv(13)(p13q14)[6]/ 46, idem, der(15)t(15;15)(p13;q21)[5]/ 46, idem, der(4)t(4;15)(q?34;q21)[4]/ 46, XY[2]	4	46, XY, der(17)t(17;17)(p11;q21)[3]/ 45, idem, add(1)(q22), del(3)(q21q26), dic(13;14)(p13;p13)inv(13)(p13q14)[8][b]/ 46, idem, der(15)t(15;15)(p13;q21)[6]/ 46, idem, der(4)t(4;15)(q?34;q21)[5]/ 45, idem, −13, dic(13;14)(p13;p13)inv(13)(p13q14)[3]/ 45, idem, −13, dic(13;14)(p13;p13)inv(13)(p13q14), add(22)(p11)[3][b]/ 46, idem, add(7)(p22)[3][b]	7
8	Yes	1	46, XX, t(11;13)(p15;q14)[10]/ 46, XX[9]	3	46, XX, t(11;13)(p15;q14)[11]/ 45, XX, dic(6;17)(q21;p13)[5][c]/ 46, XX[6]	4
10	n/a	n/a	46, XY, t(13;18)(q14;p11)[3]/ 46, XY[11]	D.O.D.	46, XY, del(5)(q15q34)[6][c]/ 46, XY, t(13;18)(q14;p11)[5]/ 46, XY[8]	15
11	n/a	4	46, XY, del(13)(q13q14)[6]/ 46, idem, t(13;19)(q14;p13)[6]/ 46, idem, add(18)(p11)	4	46, XY, del(13)(q13q14)[11]/ 46, idem, t(13;19)(q14;p13)[8]/ 46, idem, der(6)t(6;15)(q21;q21), add(15)(q21)[4][b]/ 46, XY[1]	No prog.
13	Yes	0	46, XY, del(13)(q13q22)[15]/ 46, XY[5]	3	46, XY, del(13)(q13q22)[15]/ 46, XY, del(6)(q?15q24), del(13)(q13q22)[5][b]	20
16	No	2	45, X, −Y, t(6;13)(q15;q14)[8]/ 45, idem, add(1)(q41)[9]/ 46, XY[3]	2	45, X, −Y, t(6;13)(q15;q14)[6]/ 45, idem, add(1)(q41)[14]/ 44, idem, add(1)(q41),−4, der (17)t(4;17)(q12;p13)[3][b]/ 46, XY[1]	No prog.
19	Yes	4	47, XY, +12[14]/ 94, XXYY, +12, +12[2]/ 46, XY[1]	4	47, XY, +12[8]/ 47, idem, t(14;14)(q24;q32) [11][b]/ 47, idem, t(9;9)(q34;q34) [2][b]/ 47, idem, add(9)(q34), del(14)(q24)[2][b]	No prog.
20	No	3	47, XY, +12[22]/ 46, XY[3]	D.O.D.	47, XY, +12[13]/ 47, XY, +inv(12)(p13;q24)[6][c], 46, XY[1]	20
21	Yes	0	46, XY, del(6)(q23q27), t(12;14)(p13;q21)[7]/ 46, idem, del(7)(q32q36)[3]/ 46, XY, t(1;9)(p36;q22)[8]/ 46, XY, del;(6)(q15q26)[5]/ 46, XY, del(17)(p11)[3]/ 46, XY, t(6;12)(p22;q13)[2]/ 46, XY, add(7)(q35)[2]	4	46, XY, der(17)t(17;21)(p11;q11)[2][b]/ 45, idem, −21[3][c]/ 46, XY, del(17)(p11)[10]/ 46, XY, t(1;9)(p36;q22)[7]/ 46, XY, del(6)(q23q27), t(12;14)(p13;q21)[4]/ 46, XY, del(1)(q12q44)[2][c]/ 46, XY, del(6)(q15q26)[2]/ 46, XY, del(11)(q21q24)[2][c]/ 46, XY, ?t(1;3)(q25;p25)[2][c]/ 46, XY, t(6;12)(p22;q13)[2]/ 46, XY, t(12;14)(p13;q21)[2]/ 46, XY, del(6)(q23q27), del(7)(q32q36), t(12;14)(p13;q21)[2][b]/ 46, XY[3]	8
24	Yes	0	46, XY, t(9;14)(p12;q32)[2]/ 46, XY[18]	1	45, XY, i(2)(p10), t(13;22)(q10;q10), der(17)t(2;17)(q11;q25)[5][c]/ 46, XY[15]	16
25	No	2	46, XY, der(17)t(3;17)(q23;q25)[9]/ 46, XY, i(21)(q10)[4]/ 46, XY[5]	2	46, XY, i(21)(q10)[5]/ 46, XY, i(15)(q10)[4][c]/ 46, XY, add(15)(p11)[3][c]/ 46, XY, add(21)(p11)[3][c]/ 46, XY, der(17)t(3;17)(q23;q25)[3]/ 46, XY[6]	No prog.

TABLE I. (continued)

Patient no.	Prior therapy	Baseline Rai stage	Baseline karyotype	Follow-up Rai stage	Follow-up karyotype[a]	Time to disease progression (months)
32	Yes	2	45, XY, −4, der(7)t(4;7)(q21;p22), der(14)t(14;18)(p11;q11), −18, +r[11]/ 46, XY[5]	2	46, XY, add(13)(p11), der(18)t(18;20)(p11;q12), −20, +r[4][c]/ 46, idem, del(6)(q12q26)[4][c]/ 45, XY, −4, der(7)t(4;7)(q21;p22), der(14)t(14;18)(p11;q11), −18, +r[9]/ 46, XY[3]	No prog.
33	Yes	3	46, XY[17]	3	46, XY, del(11)(q13q14)[8][c]/ 46, XY[11]	No prog.
34	Yes	0	46, XY[18]	0	46, XY, del(13)(q14q22)[7][c]/ 46, idem, t(7;8)(q22;p11)[2][c]/ 46, XY, t(3;13)(p26;q14)[3][c]/ 46, XY[8]	No prog.
36	No	0	46, XY[19]	0	46, XY, t(6;12)(p21;q13)[5][c]/ 46, XY[15]	No prog.
37	Yes	4	46, XY[22]	3	46, XY, der(5)t(5;11)(q11;q25), der(11)t(5;11)(q11;p11)inv(11)(p11q14)[12][c]/ 46, XY[7]	No prog.
42	No	1	46, XY[40]	2	45, XY, −8, der(14)t(8;14)(q12;p11)[2][c]/ 45, idem, add(4)(q34)[2][c]/ 45, idem, der(6)t(2;6)(q24;q24)[2][c]/ 46, XY, add(1)(q31), −13, add(16)(p13), +mar[2][c]/ 46, XY[5]	8
45	No	2	46, XY[19]	2	46, XY, t(2;13)(p13;q14)[3][c]/ 46, XY[17]	No prog.
46	No	0	46, XY[16]	0	46, XY, t(2;3)(p13;p13), t(8;16)(q11;q12)[11][c]/ 46, XY[9]	No prog.

*Numbers in brackets refer to number of cells exhibiting the listed karyotype. Nonclonal karyotypic abnormalities are not listed. D.O.D., Dead of disease; No prog., no advance in Rai stage over the course of the study. n/a, information not available.
[a]Table does not include additional abnormalities acquired in subsequent karyotypes for patients 2, 21, 24, and 42.
[b]Apparent evolution of pre-existing clone.
[c]Novel clone, not related to pre-existing clone.

TABLE II. Most Commonly Involved Sites of Secondarily Acquired Structural Chromosome Abnormalities in 22 B-CLL Patients in Whom Karyotypic Evolution Was Observed*

Site	Number of patients
6q	6
1q	4
3p	4
12q	4
13q14	2
Other 13q	2
11q	3[a]
17p	3

*B-CLL, B-cell chronic lymphocytic leukemia.
[a]One additional patient had an acquired nonclonal abnormality involving chromosome 11q (del(11)(q13q23)).

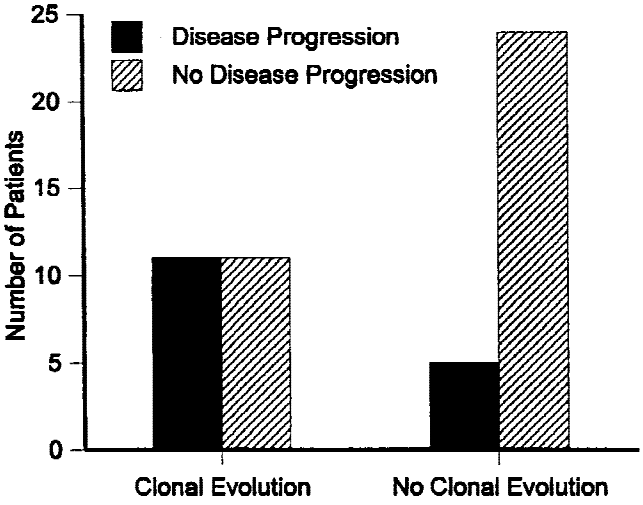

Fig. 1. The relationship between karyotypic evolution and clinical disease progression in B-CLL. Patients experiencing clinical karyotypic evolution were more likely to experience clinical disease progression (advance in Rai stage or death from disease) ($P = 0.017$ by χ^2 test).

(16%). As in the current study, a variety of secondary abnormalities were identified, without a consistent pattern of clonal evolution. Only one patient in Oscier's study acquired a structural 6q abnormality on sequential study, and that patient did not undergo clinical progression. Oscier et al. [10] also reported that the rate of disease progression was no different in patients with and without karyotypic evolution. In a more recent study, Fegan and colleagues [12] reported karyotypic evolution in 17 of 45 patients (38%), a number closer to the rate of 43% in the current study. Furthermore, Fegan et al. reported disease progression in 75% of patients with karyotypic evolution, and in only 17% of patients without karyotypic evolution. They did not specifically report secondarily acquired abnormalities of chromosome 6, but instead they demonstrated that patients who acquire abnormalities of the long arm of chromosome 11 undergo accelerated clinical disease progression. The association

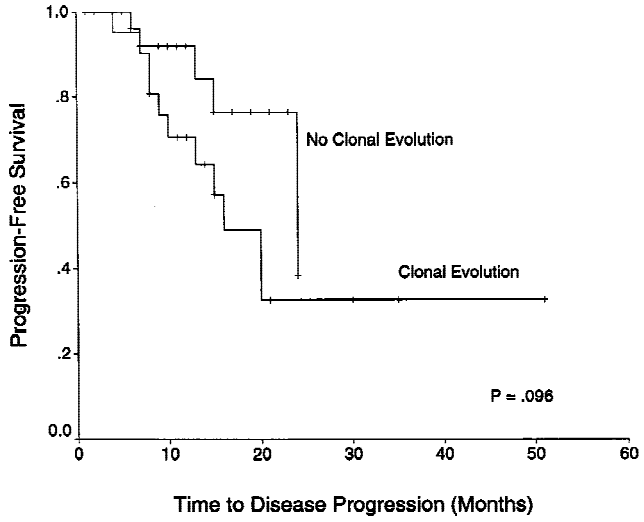

Fig. 2. Kaplan-Meier product limit curves for progression-free survival in patients with B-CLL. Karyotypic evolution was associated with shortened progression-free survival intervals, but this difference did not reach statistical significance.

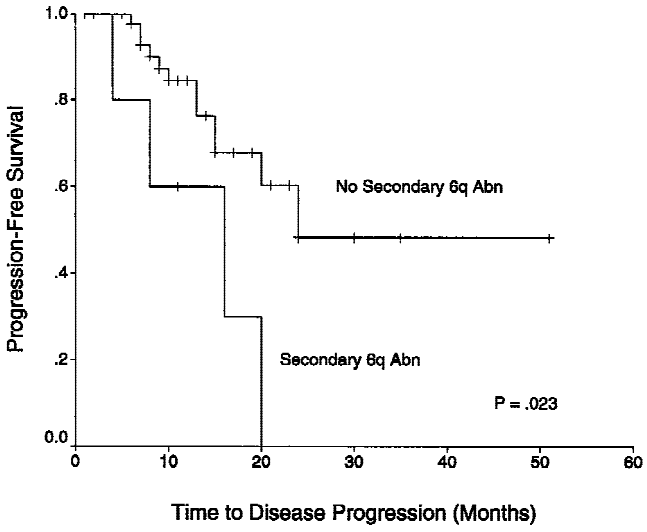

Fig. 3. Kaplan-Meier product limit curves for progression-free survival in patients with B-CLL. Patients who acquired abnormalities of chromosome 6q over time demonstrated shortened progression-free survival intervals.

of 11q abnormalities in B-CLL with aggressive disease has also been reported elsewhere in nonsequential cytogenetic studies [19]. In our cohort, only three patients developed 11q abnormalities, and one underwent clinical progression.

Ideally, the study of karyotypic evolution in B-CLL would include analysis of common cytogenetic abnormalities by fluorescence in situ hybridization (FISH) or other molecular genetic technique. Although the retrospective nature of this study did not allow for such analysis, the sequential study of B-CLL by conventional karyotyping allowed for the detection of multiple structural chromosome abnormalities that would not have been detected routinely by molecular techniques, including the six cases of acquired 6q aberrations.

In the current study, patients in whom karyotypic evolution was observed were significantly more likely to undergo advances in clinical stage over the course of the study than were patients with stable karyotypes. This relationship between karyotypic evolution and disease progression was statistically significant ($P = .017$), and is similar to the findings of Fegan and colleagues [12]. When patients with secondary 6q abnormalities were compared to patients without secondary 6q abnormalities, there was no difference in the overall likelihood of eventual clinical progression. However, the time intervals to clinical progression were significantly shorter in the group demonstrating temporal acquisition of 6q abnormalities (Figure 3). This observation supports prior data that suggest a relatively poor prognosis in B-CLL patients with 6q abnormalities [16]. Our data further suggest that this poor outcome might be related to the clonal acquisition of 6q abnormalities over time, rather than the presence of these abnormalities at baseline.

Over one-half of the patients in the current report had received antileukemic therapy before entry into the study. The potential influence of therapy on cytogenetic evolution is not entirely clear, but no overall difference was observed in karyotypic evolution between patients with and without prior treatment.

Data from previous investigations suggest that disruption of a putative tumor suppressor gene on chromosome 13q14 occurs very early in CLL leukemogenesis, and may represent the initiating genetic event [20,21]. We documented acquired structural 13q14 abnormalities in two patients. However, unlike patients with acquired 6q abnormalities, both of these patients had normal karyotypes at the time of initial study. It is possible that the structural abnormalities were present at baseline, but that the malignant cells were simply not represented in the karyotypes. This notion is supported by prior B-CLL studies that document detection of 13q14 deletions in the absence of karyotypic abnormalities by the use of fluorescent labeled DNA probes [22,23]. Interestingly, Oscier and coworkers observed acquired 13q14 abnormalities in four patients, all of whom had normal karyotypes at baseline [10].

Continued study of B-CLL clonal evolution patterns is necessary to elucidate more clearly the relevant genetic events that occur over the natural course of this disease. However, the findings in the current study indicate that clonal karyotypic evolution in B-CLL is common, and is associated with clinical disease progression. We have identified the long arm of chromosome 6 as a potentially important region in the clonal evolution of B-CLL. However, the lack of a consistent pattern of cytogenetic evo-

lution reflects the heterogeneity of B-CLL. This further suggests that B-CLL heterogeneity may be due to a series of alternative cytogenetic events that contribute differentially to disease progression in this disease.

REFERENCES

1. Peterson LC, Lindquist LL, Church S, Kay NE: Frequent clonal abnormalities of chromosome 13q14 in B-cell chronic lymphocytic leukemia: Multiple clones, subclones, and non-clonal alterations in 82 midwestern patients. Genes Chromosomes Cancer 4:273, 1992.
2. Juliusson G, Oscier DG, Fitchett M, Ross FM, Stockdill G, Mackie JJ, Parker AC, Castoldi GL, Cuneo A, Knuutila S, Elonen E, Gahrton G: Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. N Engl J Med 323:720, 1990.
3. Criel A, Wlodarska I, Meeus P, Stul M, Louwagie A, Van Hoof A, Hidajat M, Mecucci C, Van den Berghe H: Trisomy 12 is uncommon in typical chronic lymphocytic leukemias. Br J Hematol 87:523, 1994.
4. Finn WG, Thangavelu M, Yelevarthi KY, Goolsby CL, Tallman MS, Traynor A, Peterson LC: Karyotype correlates with peripheral blood morphology and immunophenotype in chronic lymphocytic leukemia. Am J Clin Pathol 105:458, 1996.
5. Que TH, Marco JG, Ellis J, Matutes E, Brito-Babapulle V, Boyle S, Catovsky D: Trisomy 12 in chronic lymphocytic leukemia detected by fluorescence in situ hybridization: Analysis by stage, immunophenotype, and morphology. Blood 82:571, 1993.
6. Kroft SH, Finn WG, Kay NE, Peterson LC: Isolated 13q14 abnormalities and normal karyotypes are associated with typical lymphocyte morphology in B-cell chronic lymphocytic leukemia. Am J Clin Pathol 107:275, 1997.
7. Hernandez JM, Mecucci C, Criel A, Meeus P, Michaux L, Van Hoof A, Veerhoef G, Louwagie A, Scheiff J-M, Michaux J-L, Boogaerts M, Van den Berghe H: Cytogenetic analysis of B cell chronic lymphoid leukemias classified according to morphologic and immunophenotypic (FAB) criteria. Leukemia 9:2140, 1995.
8. Juliusson G, Friberg K, Gahrton G: Consistency of chromosomal aberrations in chronic B-lymphocytic leukemia: A longitudinal cytogenetic study of 41 patients. Cancer 62:500, 1988.
9. Nowell PC, Moreau L, Growney P, Besa EC: Karyotypic stability in chronic B-cell leukemia. Cancer Genet Cytogenet 33:155, 1988.
10. Oscier D, Fitchett M, Herbert T, Lambert R: Karyotypic evolution in B-cell chronic lymphocytic leukemia. Genes Chromosomes Cancer 3:16, 1991.
11. Hakim I, Rechavi G, Brok-Simoni F, Grossman Z, Amariglio N, Mandel M, Ramot B, Ben-Bassat I, Katzir N: Analysis of rearranged immunoglobulin genes indicating a process of clonal evolution in chronic lymphocytic leukaemia. Br J Haematol 84:436, 1993.
12. Fegan C, Robinson H, Thompson P, Wittaker JA, White D: Karyotypic evolution in CLL: Identification of a new sub-group of patients with deletions of 11q and advanced or progressive disease. Leukemia 9: 2003, 1995.
13. Bennett JM, Catovsky D, Daniel M-T, Flandrin G, Galton DAG, Gralnick HR, Sultan C: Proposals for the classification of chronic (mature) B and T lymphoid malignancies. J Clin Pathol 42:567, 1989.
14. ISCN 1991. Cancer Cytogenetics (Supplement to an International System for Human Cytogenetic Nomenclature.) S. Karger: Basel, Switzerland, 1991.
15. Rai KR, Sawitzky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS: Clinical staging of chronic lymphocytic leukemia. Blood 46:219, 1975.
16. Philip P, Geisler C, Hansen MM, Hasselbalch H, Christensen BE, Drivsholm A, Lund B, Nielsen JB, Jensen KB, Andersen E: Aberrations of chromosome 6 in 193 newly diagnosed untreated cases of chronic lymphocytic leukemia. Cancer Genet Cytogenet 53:35, 1991.
17. Offit K, Parsa NZ, Gaidano G, Filippa DA, Louie D, Pan D, Jhanwar SC, Dalla-Favera R, Chaganti RSK: 6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma. Blood 82: 2157, 1993.
18. Johansson B, Mertens F, Mitelman F: Cytogenetic evolution patterns in non-Hodgkin's lymphoma. Blood 86:3905, 1995.
19. Dohnoer H, Stilgenbauer S, James MR, Benner A, Weilguni T, Bentz M, Fischer K, Hunstein W, Lichter P: 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood 89:2516, 1997.
20. Jabbar SAB, Ganeshaguru K, Wickremasinghe RG, Hoffbrand AV, Foroni L: Deletion of chromosome 13 (band q14) but not trisomy 12 is a clonal event in B-chronic lymphocytic leukaemia (CLL). Br J Haematol 90:476, 1995.
21. Bullrich F, Veronese ML, Kitada S, Jurlander J, Caligiuri MA, Reed JC, Croce CM: Minimal region of loss at 13q14 in B-cell chronic lymphocytic leukemia. Blood 88:3109, 1996.
22. Stilgenbauer S, Dohner H, Bulgay-Morschel M, Weitz S, Bentz, Lichter P: High frequency of monoallelic retinoblastoma gene deletion in B-cell chronic lymphoid leukemia shown by interphase cytogenetics. Blood 81:2118, 1993.
23. Huang JC, Finn WG, Yelavarthi KK, Thangavelu M, Chen PX, Peterson L: Rb-1 gene deletion as a marker of chromosome 13q14 abnormalities in B-cell chronic lymphocytic leukemia. Am J Clin Pathol 106:424, 1996.